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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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R C van Dijk



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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se référer à la description.)

An antisense oligonucleotide to inhibit "Melanoma inhibitory activity" MIA

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An antisense oligonucleotide to inhibit
"Melanoma Inhibitory Activity", MIA

Field of the invention

The present invention relates to an antisense oligonucleotide or modifications thereof for the inhibition of the expression and/or functional activity of Melanoma Inhibitory Activity "MIA", a pharmaceutical composition comprising the oligonucleotide or modifications thereof and its use for the prevention or the treatment of neoplasms, infections and /or immunosuppressive disorders.

Background

10 The polypeptide "Melanoma Inhibitory Activity" MIA, was discovered in 1989 and initially described as a factor that inhibits growth of melanoma tumor cells. The melanoma inhibitory activity (MIA) protein was identified within growth-inhibiting activities, purified from tissue culture supernatant of the human melanoma cell line HTZ-19 (Bogdahn et al., Cancer Res. 49: 5358-5363, 15 1989). Weilbach et al. further demonstrated that MIA inhibits cell proliferation by prolonging of the S-Phase and arrest of the cells in the G2 compartment (Cancer Res. 50; 6981-86. 1990). The antiproliferative action of MIA was also demonstrated in other tumor cells and Peripheral Blood Mononuclear Cells (PBMCs), where recombinant human rMIA inhibited IL-2- or PHA-induced 20 PBMCs proliferation in a dose-dependant manner. Additionally, auto- and allo-genic LAK-cytotoxicity was also inhibited by MIA (Jachimczak et al., Proceeding of AACR, 41: 115, 2000).

Blesch et al. identified MIA as a 131-amino acid precursor, processed into a mature 107-amino acid protein. This publication confirmed that MIA acts as a 25 potent tumor cell growth inhibitor for malignant melanoma cell and further extended this observation to other neuroectodermal tumors. They concluded that "... MIA ... might be attractive as a future antitumor therapeutical substance" (Cancer Res. 54; 5695-5701, 1994).

However, further studies revealed expression patterns inconsistent with a tumor suppressor. In contrast, van Groningen et al. found MIA mRNA expression in non metastasizing cell lines and an inverse correlation of MIA mRNA expression with pigmentation in melanoma metastasis lesions, but notably
5 expression was found to be absent in highly metastasizing cell lines (Cancer Res. 55; 6237-6243, 1995).

More recently, Bosserhoff et al. observed that MIA specifically inhibits attachment of melanoma cells to fibronectin and laminin thereby masking the binding sites of Integrins to these extracellular matrix components and promoting
10 invasion and metastasis in vivo (Bosserhoff et al., Hautarzt 49, 762-769, 1998; Stoll et al., EMBO J 20, 340-349, 2001). The growth-inhibitory activity in vitro may therefore reflect the ability of MIA to interfere with the attachment of cells to culture dishes observed by Blesch et al. (Cancer Res. 54; 5695-5701, 1994).

15 Further experiments performed in hamster by Guba et al. (Brit. J. Cancer 83, 1216-1222, 2000) analyzed the in vivo role of MIA during melanoma metastasis. Enforced expression of MIA in MIA-transfected A-mel3 hamster melanoma cells significantly increased their metastatic potential compared to control transfected cells. In addition, MIA overexpressing cells showed a higher rate of
20 both tumor cell invasion and extravasation. Bosserhoff et al. confirmed these results by the use of stably MIA-transfected B16 mouse melanoma cells (Bosserhoff et al., Melanoma Res. 11; 417-421, 2001). The capacity of these cells to form lung metastases in syngeneic C57Bl6 mice was strictly correlated to the level of MIA secretion in mice.

25 The clinical correlation of MIA expression with melanoma progression was discovered by Bosserhoff et al. (Cancer Res. 57; 3149-53; 1997) and confirmed by others (Stahlecker et al., Anticancer Res. 19; 2691-3, 2000). 81% of the sera obtained from patients with metastasized malignant melanomas in stage III revealed enhanced MIA values and even 97% of the sera from patients
30 in stage IV. Patients in stage I or II disease had only slightly enhanced

MIA levels of 13 and 23 %, respectively (Bosserhoff et al., Cancer Res. 57; 3149-53; 1997)

5 Additionally, there is evidence that MIA might be useful as a marker in other types of malignant tumors, since seropositivety was also reported for some patients with advanced gastrointestinal carcinoma with a very poor prognosis (Wagner et al., Med. Oncol.1735-38, 2000). Recently, MIA levels were measured in cerebrospinal fluid specimes from patients with spinal diseases (Natsume et al., Spine 26(2): 157-60, 2001).

10 The concentration of MIA in cervical myelopathy, lumbar canal stenosis, and lumbar disc herniation was significantly higher than in the control group. Interestingly, MIA is not expressed in the central nervous system under physiological conditions.

15 Furthermore, in situ-hybridization experiments, as well as Immuno histochemistry localize MIA in the developmental embryo within the growth zone of the skeletal system, and it is being expressed, secreted, and deposited around the chondrocytes. Additionally, It is pathologically expressed in chondrosarcoma (Bosserhoff et al., Dev Dyn 208(4): 516-525, 1997).

20 Since high levels of MIA were found in chondrocytes, MIA was also investigated as a potential marker for rheumatoid arthritis and cartilage damage. Muller-Ladner et al. found increased MIA serum concentrations in patients with rheumatic diseases with joint destruction, osteoarthritis, HLA-27-associated oligoarthritis, psoriatic arthritis and rheumatic arthritis, where the most significant increase in MIA was found (Muller-Ladner et al., Rheumatology 38; 148-154, 1999).

25 However, in the case of the tumors, MIA was found to be expressed and secreted into the serum by all of the malignant melanomas examined, but not in other skin tumor, including basal cell cancer and squamous cell cancer, nor in normal melanocytes and keratinocytes (Bosserhoff et al., J Pathol 187(4): 446-454, 1999). Additionally, MIA expression was further found in all ad-

vanced stage breast cancer and metastases analyzed suggesting a much broader expression of MIA in malignant epithelial neoplasms than previously determined by serum studies (Bosserhoff et al., J Pathol 187(4): 446-454, 1999).

5 **Prior art**

Molecules for the inhibition of the expression and/or functional activity of MIA are published in WO 01/68122. The inhibition is achieved by using at least one nucleic acid molecule, peptide, protein or low molecular weight substance, wherein the nucleic acid molecule is an oligo- or polynucleotide molecule, in
10 particular an antisense molecule and/or ribozyme. The above mentioned patent-application relates to molecules including oligonucleotides able to inhibit the expression and/or function of MIA and thereby reversing the immunosuppression (WO 01/68122).

It was the object of the present invention to develop MIA antisense oligonucleotides with higher efficacy.
15

Summary of the invention

The present invention relates to an antisense-oligonucleotide with the following sequence 5' - TTG CAT AAA CCC AAG GAG - 3', modifications thereof, parts of the antisense oligonucleotide with at least 8 nucleotides and/or modifications
20 thereof for a surprisingly much more effective inhibition of the expression and/or function of "Melanoma Inhibitory Activity" MIA, thereby eliciting a more effective inhibition of tumor invasion and/or inhibition of metastasis and for a more effective stimulation of immune cells and/or the immune system than antisense-oligonucleotides of the prior art. The present invention also pertains
25 to a pharmaceutical composition comprising at least one of the antisense oligonucleotides or modifications thereof and to its use for the prevention or the treatment of neoplasms, infections and /or immunosuppressive disorders.

Although a number of oligonucleotides have already been tested so far (see WO 01/68122), the antisense oligonucleotide with the sequence 5' - TTG CAT AAA CCC AAG GAG - 3' surprisingly showed the strongest inhibition of MIA compared to the antisense oligonucleotides of the patent application WO 01/68122 with the Sequence-ID-No's 1-8.

Detailed Description

In one embodiment of the invention, the antisense oligonucleotide having the sequence 5' - TTG CAT AAA CCC AAG GAG or modifications thereof has a DNA- or RNA-type structure able to hybridize to an area of the gene region coding MIA and thereby reducing and/or inhibiting the expression of MIA. It is also understood by persons skilled in the art that fragments having subsequences of the above given antisense oligonucleotide with at least 8 nucleotides or modifications thereof work according to the invention so long as production of MIA is reduced or inhibited.

In the following, the antisense oligonucleotide with the sequence 5' - TTG CAT AAA CCC AAG GAG and antisense oligonucleotides representing parts of the this sequence with at least 8 nucleotides are referred to as the antisense oligonucleotides.

In another embodiment the antisense oligonucleotides are modified at one or more of the sugar moieties, the bases and/or the internucleotide linkages as well as the phosphate moieties. For example, the modifications of the oligonucleotides comprise modifications such as phosphorothioate (S-ODN) internucleotide linkages, methylphosphonate internucleotide linkages, phosphoramidate linkages, peptide linkages, 2'-O-alkyl modifications of the sugar, in particular methyl, ethyl, propyl, butyl and the like, 2'-methoxyethoxy modifications of the sugar and/or modifications of the bases. The various modifications may be combined in one oligonucleotide.

In a preferred embodiment the antisense-oligonucleotides or an effective modification thereof is a phosphorothioate-oligodeoxynucleotide.

Methods for synthesizing antisense-oligonucleotides of the invention are known to those skilled in the art. The principle of one possible synthesis method employs solid phase synthesis using phosphite triester chemistry by growing the nucleotide chain in 3' -> 5' direction and is described in the following:

- 5 The respective nucleotide is coupled to the first nucleoside, which is covalently attached to the solid phase comprising the steps of
 - a) cleaving 5'DMT protecting group of the previous nucleotide
 - b) adding the respective nucleotide for chain propagation
 - c) modifying the phosphite group and subsequently cap unreacted 5'-
10 hydroxyl groups
 - d) repetitive cycles of a) to-c) for the full length oligonucleotide
 - e) cleaving the oligonucleotide from the solid support
 - f) followed by working up the synthesis product.

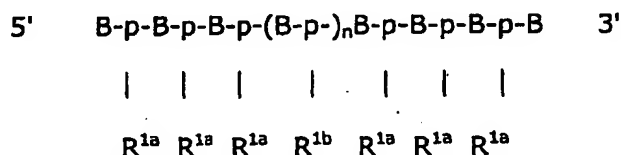
The chemical structure of oligonucleotides and their modifications are given in
15 Figure 2. The oligonucleotide chain is to be understood as a detail out of a longer nucleotide chain.

In Figure 2a lit. B means an organic base such as adenine (A), guanine (G), cytosine (C) or thymine (T) (shown in Figure 2b) in oligodeoxy-ribonucleotides and the bases adenine (A), guanine (G), cytosine (C), uracile (U) (shown in
20 Figure 2c) in oligo-ribonucleotides. The bases are coupled via the nitrogen N9 in the case of the bases A and G or the nitrogen N1 in the case of the bases C, T and U to the desoxyribose or ribose, respectively. The sequence of the bases is the reverse complement of the genetic target sequence MIA.

The modifications of oligodeoxy-ribonucleotides and oligo-ribonucleotides in
25 Figure 2a are as follows, whereby both, oligodeoxy-ribonucleotides and oligo-ribonucleotides are referred to as oligonucleotides:

1. Oligonucleotides where all R^1 are substituted by
 - 1.1 $R^1 = O^-M^+$, where all M^+ is Na^+ or H^+
 - 1.2 $R^1 = S^-M^+$, where all M^+ is Na^+ or H^+
 - 1.3 $R^1 = CH_3$
 - 5 1.4 $R^1 = C_2H_5$
 - 1.5 $R^1 = OCH_3$
 - 1.6 $R^1 = OC_2H_5$
2. Oligonucleotides where R^1 is varied at the internucleotide phosphates within one oligonucleotide. In the following formula, R^1 of Figure 2a) is referred to as R^{1a} or R^{1b} .

10



where

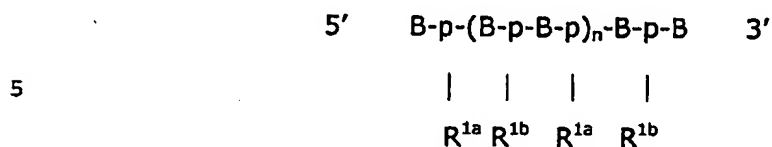
- 15 B = one of the bases A, C, G or T in oligodeoxy-ribonucleotides or accordingly the bases A, C, G or U in oligo-ribonucleotides

p = internucleotide phosphate

(B-p)_n = an oligodeoxy-ribonucleotide or oligo-ribonucleotide stretch wherein n = 2 - 12

- 20 2.1 $R^{1a} = S^-M^+$, where all M^+ is Na^+ or H^+
 $R^{1b} = O^-M^+$, where all M^+ is Na^+ or H^+
- 2.2 $R^{1a} = CH_3$
 $R^{1b} = O^-M^+$, where all M^+ is Na^+ or H^+
- 2.3 $R^{1a} = S^-M^+$, where all M^+ is Na^+ or H^+
 $R^{1b} = CH_3$
- 25 2.4 $R^{1a} = CH_3$
 $R^{1b} = S^-M^+$, where all M^+ is Na^+ or H^+

3. Oligodeoxy-ribonucleotides where R^1 is alternated at the internucleotide phosphates within one oligonucleotide. In the following formula, R^1 of Figure 2a) is referred to as R^{1a} or R^{1b} .



where

B = one of the bases A, C, G or T comprised in oligodeoxy-ribonucleotides or accordingly the bases A, C, G or U comprised in oligo-ribonucleotides depending on gene sequence

p = internucleotide phosphate

$(B-p-B-p)_n$ = an oligodeoxy-ribonucleotide or oligo-ribonucleotide stretch wherein $n = 3 - 8$

3.1 $R^{1a} = S^-M^+$, where all M^+ is Na^+ or H^+
 15 $R^{1b} = O^-M^+$, where all M^+ is Na^+ or H^+

3.2 $R^{1a} = CH_3$
 $R^{1b} = O^-M^+$, where all M^+ is Na^+ or H^+

3.3 $R^{1a} = S^-M^+$, where all M^+ is Na^+ or H^+
 $R^{1b} = CH_3$

20 4. Oligonucleotides or modifications thereof, where R^2 is H or covalently coupled cholesterol, poly (L)lysine, transferrin.

5. Oligonucleotides or modifications thereof, where R^3 is H or covalently coupled cholesterol, poly (L) lysine, transferrin.

6. Any of the modifications described in 1.1 - 1.6; 2.1 - 2.4; 3.1 - 3.3; 4, 5
 25 where all R^4 are substituted by:

- 6.1 $R^4 = H$
- 6.2 $R^4 = F$
- 6.3 $R^4 = CH_3$
- 6.4 $R^4 = C_2H_5$
- 5 6.5 $R^4 = OH$
- 6.6 $R^4 = OCH_3$
- 6.7 $R^4 = OC_2H_5$

Modifications of the antisense-oligonucleotides are advantageous since they are not as fast destroyed by endogeneous factors when applied, as this is valid for
10 naturally occurring nucleotide sequences. However, it is understood by the skilled person that also naturally occurring oligonucleotides having one of the disclosed sequences can be used according to the invention. In a very preferred embodiment the modification is a phosphorothioate modification.

Among several synthesis methods for oligodeoxy-nucleotides known to those
15 skilled in the art, one possible method is described as an example in a greater detail as follows.

Oligodeoxy-nucleotides are synthesized stepwise by 5' addition of protected nucleotides using phosphite triester chemistry. The nucleotide A is introduced as
20 5'-dimethoxytrityl-deoxyadenosine (N^4 -benzoyl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite; the nucleotide C is introduced by a 5'-dimethoxytrityl-deoxycytidine(N^4 -benzoyl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite; the nucleotide G is introduced as 5'-dimethoxytrityl-deoxyguanosine(N^8 -isobutyryl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite and the nucleotide T is introduced
25 as 5'-dimethoxytrityl-deoxythymidine-N,N'-diisopropyl-2-cyanoethyl phosphoramidite. The nucleotides are preferably applied in 0.1 M concentration dissolved in acetonitrile.

Synthesis can be performed for example on controlled pore glass particles of approximately 150 nm diameter (pore diameter 500 Å) to which the 3' nucleoside is covalently attached via a long-chain alkylamin linker (average loading 30

μmol/g solid synthesis support).

The solid synthesis support is loaded into a cylindrical synthesis column, capped on both ends with filters permitting adequate flow of reagents but hold back the solid synthesis support. Reagents are delivered and removed from the synthesis column using positive pressure of inert gas. The nucleotides are added to the growing oligonucleotide chain in 3' -> 5' direction. Each nucleotide is coupled using one round of the following synthesis cycle:

Cleavage of the 5'DMT (dimethoxytrityl) protecting group of the previous nucleotide is performed with 3-chloroacetic acid in dichloromethane followed by washing the column with anhydrous acetonitrile. Then one of the bases is added in form of their protected derivative depending on the sequence plus tetrazole in acetonitrile. After reaction the reaction mixture is withdrawn and the phosphite is oxidized with a mixture of sulfur (S₈) in carbon disulfide / pyridine / triethylamine. After the oxidation reaction the mixture is withdrawn and the column is washed with acetonitrile. The unreacted 5'-hydroxyl groups are capped with simultaneous addition of 1-methylimidazole and acetic anhydride / lutidine / tetrahydrofuran. Thereafter, the synthesis column is washed with acetonitrile and the next cycle is started.

The work up procedure and purification of the synthesis products can be carried out as follows:

After the addition of the last nucleotide the deoxynucleotides are cleaved from the solid support by incubation in ammonium solution. Exocyclic base protecting groups are removed by further incubation in ammonia. Then the ammonium is evaporated under vacuum. Full-length synthesis products still bearing the 5'DMT protecting group are separated from shorter failure contaminants using reverse phase high performance liquid chromatography (HPLC) on reversed phase C₁₈ stationary phase. Eluents from the product peak are collected, dried under vacuum and the 5'-DMT protecting group cleaved by incubation in acetic acid, which is evaporated thereafter under vacuum. The synthesis products are solved in deionized water and extracted three times with diethylether. Then the products

are dried in vacuum. Another HPLC-AX (HPLC - anion exchange) chromatography is performed and the eluents from the product peak are dialysed against excess of Tris-buffer as well as a second dialysis against deionized water. The final products are lyophilized and stored dry.

- 5 It is understood that this is one possible method for the synthesis of phosphorothioate-oligodeoxynucleotide. It is clear that improvements and modifications to the description given herein will occur to those skilled in the art and will still fall within the scope of the invention.

10 Methods for the synthesis of oligo-ribonucleotides are known to those skilled in the art. One possible method using 2'-ACE RNA Chemistry is described by Scaringe, S.A.: "RNA Oligonucleotide Synthesis via 5'-Silyl-2'-Orthoester Chemistry", Methods 23, 206-217, 2001)

15 It is understood that this is one possible method for the synthesis of oligo-ribonucleotides. It is clear that improvements and modifications to the description given herein will occur to those skilled in the art.

In one embodiment, the antisense oligonucleotides or modifications thereof can be used for the stimulation of the immune system by inhibiting expression and/or functional activity of "Melanoma Inhibitory Activity" MIA.

20 In another embodiment, the antisense oligonucleotides or modifications thereof are used in a combination with at least one of the molecules, viruses, peptides and/or cell extracts listed under a) to m:

- 25 a) molecules selected from the group comprising chemokines, including lymphotactin and/or immune cell attracting factors;
- b) viruses and/or parts of viruses, including adeno-viruses, papilloma viruses, Epstein-Barr-Viruses, viruses that are non-pathogenic including Newcastle-Disease virus, Cow-pox-virus;
- c) autologous and/or heterologous MHC-molecules;
- d) molecules involved in antigen processing;
- e) molecules involved in antigen presentation;

- f) molecules involved in mediating immune-cell effects;
- g) molecules involved in mediating immune cell cytotoxic effects;
- h) molecules involved in antigen transportation;
- i) co-stimulatory molecules;
- 5 j) peptides enhancing recognition by immune cell and/or cytotoxic effects of immune cells;
- k) peptides containing one or more amino acids differing between a protein in the target cell from the other cells within an organism including, but not limited to antigens, specific for melanoma cells and/or melanocytes and/or breast cells and/or breast cancer cells; according to the invention the inhibition of the syntheses and/or function of MIA is achieved by using molecule of group i) wherein
- 10 l) molecules selected from the group comprising the peptides according to
- j) being
- 15 • peptides containing one or more mutations and/or amino acid substitutions of the Ras protein amino, the p53 protein, the EGF receptor protein, fusion peptides and/or fusion proteins, the retinoblastoma protein, peptides containing one or more mutations and/or amino acid substitutions and/or amino acid substitutions caused by gene rearrangements and/or gene translocations, peptides containing one or
- 20 more mutations and/or amino acid substitutions of proteins coded by oncogenes and/or protooncogenes, proteins coded by anti-oncogenes and/or tumor suppressor genes;
- 25 • peptides derived from proteins differing in the target cell by one or amino acids from the proteins expressed by other cells in the same organism,
- peptides derived from viral antigens and/or coded by viral nucleic acids
- 30 • peptides derived from proteins over expressed in the target cell compared to a normal cell

-  combinations thereof

m) tumor cell extracts and/or tumor cell lysates and/or adjuvants.

The molecules, including at least one antisense oligonucleotide, viruses, peptides and/or cell extracts listed under a) to m) can be applied by, to the following methods, whereby application is not limited to these methods:

- by vaccination of a human organism with DNA and/or RNA coding for all or part of the molecules listed under a) to m) and/or polypeptides contained in the molecules listed under a) to m)
 - by transfection of an human organism and/or transfecting the target cells and/or target pathogens with genes coding for the molecules listed under a) to m)
 - parenteral by intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion and/or locally by oral, epidermal or transdermal administration
- 15 In a further embodiment, the antisense oligonucleotides or modifications thereof can be combined with an immunostimulatory agent, selected from the group of cytokines and/or inhibitors of the expression and/or function of interleukin-10 and/or transforming growth factor beta (TGF- β) and/or Prostaglandin B2 and/or receptors for Prostaglandin E2 and/or inhibitors of VEGF.
- 20 In another embodiment, the antisense oligonucleotides or modifications thereof can be combined with at least one of the following molecules, methods, peptides and/or cell extracts:
- molecules enhancing the immune response against diseased cells or pathogens
 - methods and/or molecules enhancing immunogenicity of target cells and/or target pathogens

- immunostimulatory molecules, comprising cytokines including Interleukins, such as IL-1, IL-2, IL-4, IL- 12, IL-18
- methods for enhancing the expression of cytokines in target cells or pathogens by stimulating their expression and/or by transfecting expression systems into the target cell or target pathogen, capable of expressing these cytokines and/or
- chemokines attracting immune cells including lymphotactin
- methods for enhancing the expression of chemokines in target cells or pathogens by stimulating their expression and/or by transfecting expression systems into the target cell and/or target pathogen, capable of expressing these chemokines
- peptides and/or DNA and/or RNA molecules and or other antigens that are found in tumor cells and/or pathogens, but not in normal cells
- methods for enhancing the expression of peptides and/or antigens that are found in tumor cells and/or pathogens, but not in normal cells
- tumor cell extracts and/or tumor cell lysates and/or adjuvants.

These molecules, including at least one antisense oligonucleotide, peptides and/or cell extracts can be applied parenteral by intravenous, intraarterial, intraperitoneal, subcutaneous or intramuscular injection or infusion and/or locally by oral, epidermal, intradermal- and transdermal administration. Preferred subjects for application are mammals, most preferred subjects are humans.

In another embodiment, the coding sequences of the antisense oligonucleotides are integrated into a DNA delivery system, comprising viral and/or non-viral vectors together with lipids selected from the group of anionic lipids, cationic lipids, non-cationic lipids and mixtures thereof.

The antisense oligonucleotides thus may contain flanking sequences and/or vector sequences and/or sequences enhancing the expression and/or transfection of the nucleic acid molecules.

5 In a preferred embodiment of the invention the coding sequences of the antisense oligonucleotides are part of one or more vectors and/or viral sequences and/or viral vectors.

10 In another embodiment of the invention the antisense oligonucleotides or modifications thereof are coupled to or mixed with folic acid, hormones, steroid hormones such as oestrogene, progesterone, corticosteroids, mineral corticoids, peptides, proteoglycans, glycolipids, phospholipids and derivatives thereof.

In a yet further embodiment, the antisense oligonucleotides or modifications thereof can be used for the preparation of a medicament.

15 In a more preferred embodiment, the antisense oligonucleotides is combined with one or more of the afore mentioned immunostimulatory agents for the preparation of a medicament, wherein the medicament can be applied locally or systemically to a tumor or other pathologically affected site or organ.

20 In another embodiment, the medicament is used for the prevention or the treatment of neoplasms and disorders selected from the group of melanoma, gastrointestinal carcinoma, breast cancer, pancreatic cancer, ovarian carcinoma, chondrosarcoma, spinal diseases, cervical myelopathy, lumbar canal stenosis, lumbar disc herniation, rheumatoid arthritis, osteoarthritis, HLA-27-associated oligoarthritis, psoriatic arthritis and rheumatic arthritis, cartilage damage and/or joint destruction.

25 Figure 1 discloses the inhibition of MIA expression by different oligonucleotides in HTZ-19 melanoma cells. The bars indicate residual MIA expression of antisense oligonucleotide treated compared to untreated medium control (Medium) or Lipofectin-treated cells (Lipofectin). The numbers 1-8 correspond

to the state of art antisense oligonucleotides of the patent application WO 01/68122 having the Sequence ID-No's 1-8 or to the antisense oligonucleotide of the present invention with the sequence 5' - TTG CAT AAA CCC AAG GAG - 3', referred to as "new". The strongest inhibition was achieved with the "new" antisense oligonucleotide being able to inhibit MIA-expression by 84% compared to the state of art antisense oligonucleotides 1-8 (corresponding to Sequence ID No's 1-8 of the patent application WO 01/68122), where inhibition of MIA-expression varied between 48% and 65%.

Figure 2a-c discloses the structure of oligonucleotides.

10 **Example**

Example 1

Inhibition of MIA-secretion by antisense oligonucleotides

The efficacy on MIA-inhibition of the antisense oligonucleotide of this invention was compared to those of the state of art which are described in WO 01/68122 with the Sequence-ID-No's 1-8. Therefore, melanoma cells were transfected with oligonucleotides and the MIA-expression in the cell culture supernatant of the cells was measured by enzyme-linked immunosorbent assay.

On day 0, 75.000 cells were seeded in 1ml medium (DMEM/10% FCS) in a 12-well tissue-culture plate. On days 1 and 2, the cells were transfected with 200 nM of antisense oligonucleotide using Lipofectin (Gibco BRL, Eggenstein, Germany) according to the manufacturers protocol. Each antisense oligonucleotide was transfected in triplicates. At the end of the 2nd transfection on day 2, the cells were cultured in DMEM/10% FCS containing 5 µM of the respective antisense oligonucleotide for further three days. The supernatants were taken and stored at -20°C until quantification of MIA-Protein.

MIA concentrations in the supernatant were measured employing ELISA according to the manufacturers protocol (Roche, Boehringer Mannheim, Germany).

Results:

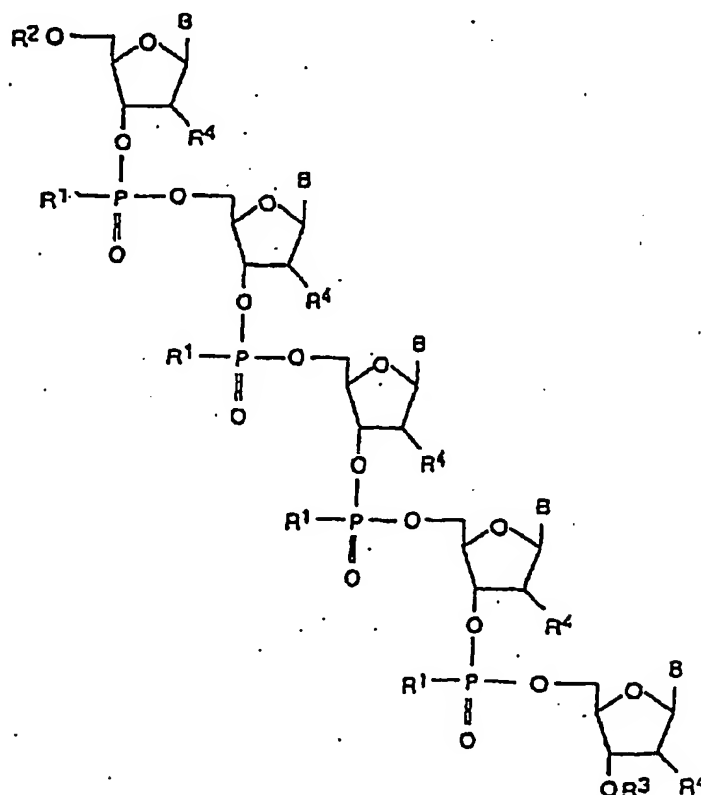
The strongest inhibition of MIA expression in the supernatant compared to untreated control-cells was achieved with the "new" antisense oligonucleotide of the present invention being able to inhibit MIA-expression by 84% compared to the state of art antisense oligonucleotide described in WO 01/68122 5 having the Sequence-ID-No's 1-8, where inhibition of MIA-expression varied between 48% and 66%.

Claims

1. An antisense oligonucleotide selected from the group of
 - the sequence 5'- TTG CAT AAA CCC AAG GAG - 3' and modifications thereof
 - 5 - a fragment having at least 8 nucleotides of the sequence 5'- TTG CAT AAA CCC AAG GAG - 3' and modifications thereof.
2. The antisense-oligonucleotide according to claim 1 wherein the modification concerns one or more of the sugar moieties, the bases and/or the internucleotide linkages and/or coupling the oligonucleotide to an enhancer of uptake and/or inhibitory activity.
3. The antisense-oligonucleotide according to claim 2 wherein the antisense-oligonucleotide is a phosphorothioate oligodeoxynucleotide.
4. The antisense-oligonucleotides according to claims 1 - 3 with respective structure:

15

20



wherein

- B = the bases A, C, G or T in oligodeoxy-ribonucleotides or accordingly the bases A, C, G or U in oligo-ribonucleotides

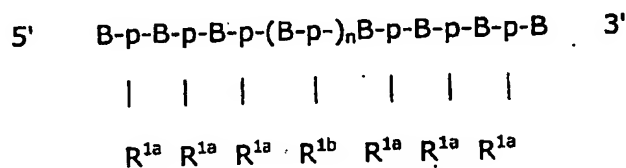
- $R^1 = O^-M^+$ ($M^+ = Na^+$ or H^+), S^-M^+ ($M^+ = Na^+$ or H^+), CH_3 , C_2H_5 , OCH_3 , OC_2H_5

5 - R^2 and/or R^3 are covalently coupled cholesterol, poly(L)lysine, transferrin or H

- $R^4 = H$, F, CH_3 , C_2H_5 , OH, OCH_3 , OC_2H_5

and the structure is to be understood as a detail out of a longer nucleotide chain.

10 5. Antisense oligonucleotides according to one of claims 1 to 4 with the formula



where

15 B = the bases A, C, G or T in oligodeoxy-ribonucleotides or accordingly the bases A, C, G or U in oligo-ribonucleotides

p = internucleotide phosphate

$(B-p)_n =$ an oligodeoxy-ribonucleotide or oligo-ribonucleotide stretch wherein $n = 2 - 12$

20 and where R^1 , referred to as R^{1a} or R^{1b} , is varied at the internucleotide phosphates within one oligonucleotide:

$R^{1a} = S^-M^+$, where all M^+ is Na^+ or H^+ and $R^{1b} = O^-M^+$, where all M^+ is Na^+ or H^+

or

$R^{1a} = CH_3$ and $R^{1b} = O^-M^+$, where all M^+ is Na^+ or H^+

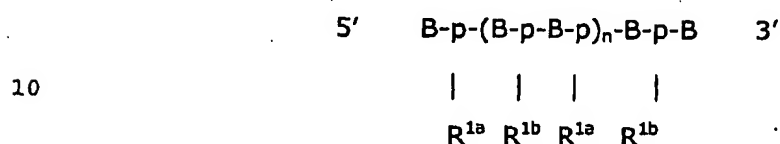
or

$R^{1a} = S^-M^+$, where all M^+ is Na^+ or H^+ and $R^{1b} = CH_3$

5 or

$R^{1a} = CH_3$ and $R^{1b} = S^-M^+$, where all M^+ is Na^+ or H^+

6. Antisense oligonucleotides according to any one of claims 1 to 5 with the formula



where

15 B = one of the bases A, C, G or T comprised in oligodeoxy-ribonucleotides or accordingly the bases A, C, G or U comprised in oligo-ribonucleotides depending on gene sequence

p = internucleotide phosphate

(B-p-B-p)_n = an oligodeoxy-ribonucleotide or oligo-ribonucleotide stretch wherein n = 3 - 8

20 and where R^1 is alternated at the internucleotide phosphates within one oligonucleotide:

$R^{1a} = S^-M^+$, where all M^+ is Na^+ or H^+ and $R^{1b} = O^-M^+$, where all M^+ is Na^+ or H^+

or

$R^{1a} = CH_3$ and $R^{1b} = O^-M^+$, where all M^+ is Na^+ or H^+

or

$R^{1a} = S^-M^+$, where all M^+ is Na^+ or H^+ and $R^{1b} = CH_3$

7. Use of the antisense-oligonucleotides according to claims 1-6 for the inhibition of the expression and/or functional activity of MIA and/or reducing invasion and/or metastasis and/or stimulating immune cells and/or the immune system.
8. A pharmaceutical composition comprising an antisense-oligonucleotide according to one of the claims 1-7.
9. The pharmaceutical composition according to claim 8 wherein the antisense-oligonucleotide is integrated into a DNA delivery system comprising viral and/or non-viral vectors together with lipid acids or derivatives thereof selected from the group of anionic lipids, cationic lipids, non-cationic lipids and mixtures thereof.
10. The pharmaceutical composition according to one of the claims 8-9 comprising additionally an immunostimulatory agent.
11. The pharmaceutical composition according to claim 10 wherein the additionally immunostimulatory agent is selected from the group of cytokines, inhibitors of the expression and/or function of interleukin-10 and/or transforming growth factor beta (TGF- β) and/or Prostaglandin B2 and/or receptors for Prostaglandin E2 and/or inhibitors of VEGF.
12. The pharmaceutical composition according to one of the claims 8-11 for the preparation of a medicament for the prevention and/or the treatment of neoplasms, infections and/or immunosuppressive disorders.
13. The use of the pharmaceutical composition according to one of the claims 8-11 for the preparation of a medicament for the prevention and/or the

treatment of disorders, neoplasms, infections and/or immunosuppressive disorders where an abnormal expression of MIA plays a role.

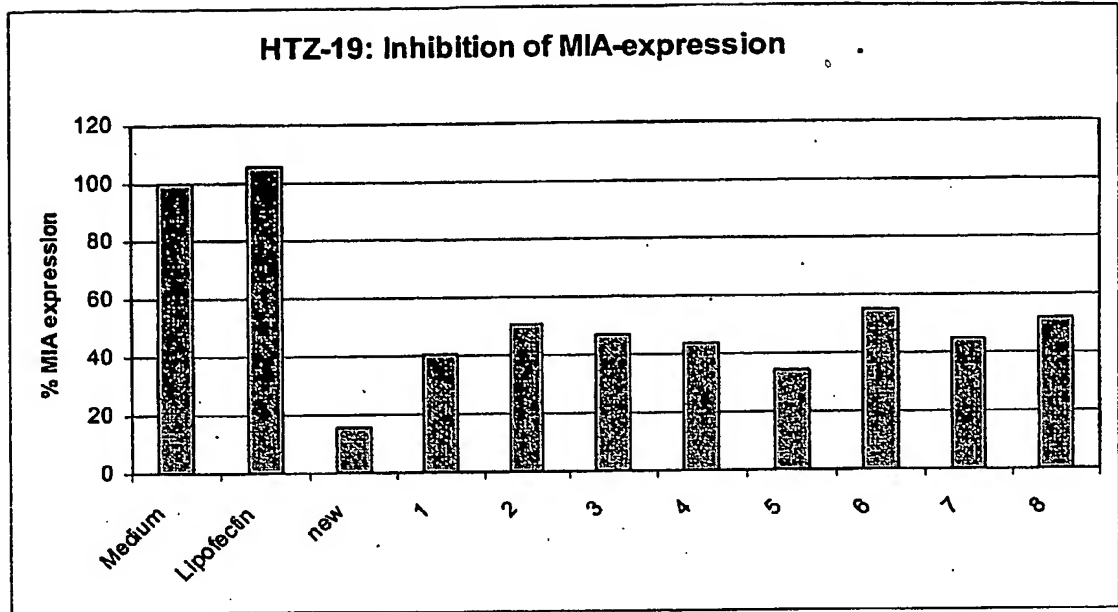
14. The use of the pharmaceutical composition according to the claims 8-11 for the preparation of a medicament for the prevention or the treatment of neoplasms and/or disorders selected from the group of melanoma, gastrointestinal carcinoma, breast cancer, pancreatic cancer, ovarian carcinoma, chondrosarcoma, spinal diseases, cervical myelopathy, lumbar canal stenosis, lumbar disc herniation, rheumatoid arthritis, osteoarthritis, HLA-27-associated oligoarthritis, psoriatic arthritis and rheumatic arthritis, cartilage damage or joint destruction.

Abstract

An antisense oligonucleotide selected from the group of

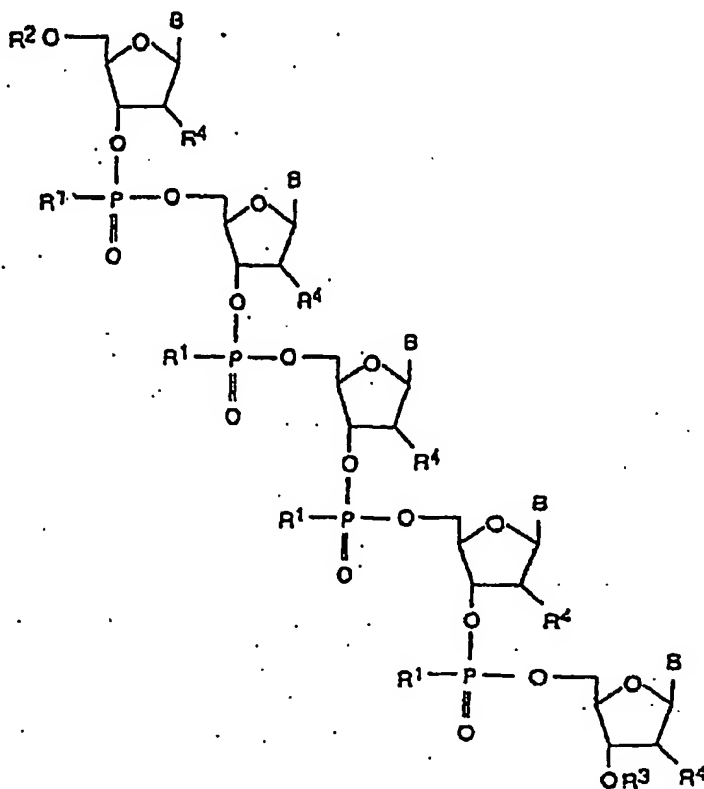
- the sequence 5'- TTG CAT AAA CCC AAG GAG - 3' and modifications thereof
- 5 - a fragment having at least 8 nucleotides of the sequence 5' - TTG CAT AAA CCC AAG GAG - 3' and modifications thereof.

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5 Figur 1

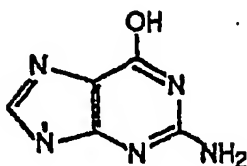
Figure 2 a)



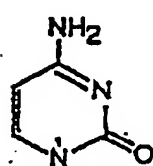
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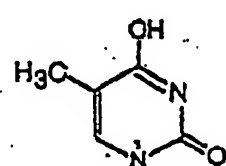
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Guanin

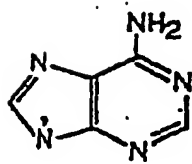


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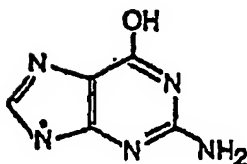


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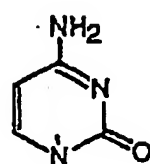
c)



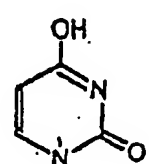
Adenin



Guanin



Cytosin



Uracil

SEQUENCE LISTING

EPO-Munich
58
12 Aug. 2003

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Inhibitory Activity": MIA

<130> Antisense

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